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## Lymphocyte to monocyte ratio (LMR) predicts mortality in patients with liver cirrhosis

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3 **Lymphocyte to monocyte ratio (LMR) predicts mortality in patients with liver**  
4 **cirrhosis**  
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## Abstract

**Objective:** Infection with hepatitis B virus (HBV) remains a major cause of liver cirrhosis (LC) in China. Recent reports suggest that the lymphocyte to monocyte ratio (LMR) is a potential biomarker for predicting clinical outcomes. In our study, we investigated if LMR can be used as a prognostic marker of mortality in LC patients.

**Design:** A cross-sectional study.

Setting: HBV-infected patients with LC and patients with chronic hepatitis B infection (CHB) from Department of Infectious Disease were enrolled in our retrospective cohort and 240 healthy individuals were recruited in healthcare centre in the First Affiliated Hospital of Zhejiang University.

**Participants:** 479 HBV-infected patients with LC, 134 patients with CHB, and 240 healthy individuals were enrolled.

**Primary and secondary outcome measures:** The receiver operating characteristic (ROC) curve and multivariable logistic regression analysis after adjusting gender, total protein, albumin, total bilirubin and the model for end-stage liver disease (MELD) score, were used to evaluate the power of LMR for predicting mortality following one year in LC patients.

**Results:** In LC patients, the LMR was statistically lower. The MELD score and mortality were statistically higher than those with chronic hepatitis B (CHB) and control groups. LMR in LC correlated with MELD score ( $r = 0.323$ ). The area under the ROC curve (AUROC) of LMR for predicting mortality LC was 0.789 (95% confidence interval (CI): 0.735-0.842;  $P < 0.001$ ); the AUROC of 1/LMR+MELD score was 0.885 (95% CI: 0.842-0.928;  $P < 0.001$ ), and the multivariate logistic regression analysis showed that LMR was an independent predictive factor of mortality in LC (odds ratios [OR]: 2.347, 95% CI: [1.134-4.859];  $P = 0.022$ ).

**Conclusion:** Our results strongly suggest that low LMR can be considered as an independent biomarker for predicting mortality in patients with LC.

**Keywords:** liver cirrhosis; lymphocyte to monocyte ratio; the model for end-stage liver disease score

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**Strengths and limitations of this study**

- LMR was lower in LC group, especially in the non-surviving group, compared to the control group and the CHB group.
- LMR was closely correlated to the MELD score.
- When LMR and MELD score were combined, the power for predicting mortality of LC patients were increased.
- Low LMR levels were independent factors for predicting mortality in LC patients.
- This was a retrospective study and validation cohort was lack.

For peer review only

## 68 Introduction

69 Liver cirrhosis (LC) is a common hepatic disease in China, and represents an  
70 increasing cause of morbidity and mortality<sup>1,2</sup>. Hepatitis B virus (HBV) infection  
71 remains a major cause of LC in China, with a 3% yearly incidence of decompensated  
72 cirrhosis<sup>3</sup>. Systemic inflammatory response syndrome (SIRS) is relatively common in  
73 patients with complicated cirrhosis<sup>4,5</sup>. SIRS can further deteriorate liver function,  
74 maximize the risk of complications and increase the mortality rate of LC patients<sup>4,5</sup>.  
75 SIRS is usually measured by peripheral blood count-based parameters, such as  
76 neutrophils, lymphocytes, monocytes, red blood cell distribution width (RDW), mean  
77 platelet volume (MPV) or platelet count. These parameters have been reported to be  
78 independent predictive markers of clinical outcome in cancer and different states of  
79 HBV-related hepatic disorders<sup>6-10</sup>. Among these inflammatory parameters, the  
80 neutrophil-lymphocyte ratio (NLR), RDW and monocyte ratio have been proposed as  
81 easily accessible and reliable markers<sup>6-8,11</sup>. Several recent studies suggest that the  
82 lymphocyte to monocyte ratio (LMR) is a cheap, readily available and reproducible  
83 test with a potential for predicting clinical outcomes of patients with solid tumors and  
84 hematologic malignancy, including nasopharyngeal carcinoma, colorectal cancer,  
85 pancreatic cancer, and lymphoma<sup>12-15</sup>. Moreover, Merakoulias *et al.*, found that, in  
86 90% of patients who had influenza virus, lymphopenia and/or monocytosis, LMR  
87 could be used as a time-saving and cost-effective screening test for influenza virus  
88 infection, leading to early antiviral treatment and a decreased incidence of  
89 complications<sup>16</sup>. Assuming that there may be association between LMR and LC  
90 severity, we investigated the potential prognostic value of LMR as a biomarker in  
91 HBV-related LC.

92 To the best of our knowledge, there is no data on LMR as a LC diagnostic measure for  
93 LC. We therefore investigated, in a retrospective cohort, the association between  
94 LMR in peripheral blood in LC patients, with special emphasis on the value of LMR  
95 for predicting the mortality of LC patients.

## 97 Subjects and Methods

### 99 Subjects

100 There were 134 patients with chronic hepatitis B infection (CHB) and 479 patients  
101 with HBV-related liver cirrhosis (LC) from the Department of Infectious Disease, The  
102 First Affiliated Hospital, School of Medicine, Zhejiang University, between October  
103 2012 and October 2013, included in our retrospective cohort study. CHB and LC were  
104 diagnosed according to the criteria of the 2000 Xi'an viral hepatitis management  
105 scheme<sup>17</sup>. The LC group was subdivided into two subgroups according to mortality at  
106 one year of follow up. Ninety-two LC patients died of upper gastrointestinal bleeding,  
107 hepatic encephalopathy, hepatorenal syndrome, infection, gastrointestinal bleeding  
108 and/or hepatic encephalopathy<sup>18-19</sup>. Two hundred and forty healthy controls  
109 corresponded to HBsAg negative individuals with normal liver function, normal renal  
110 function and no infection. Patients with a concurrent infection of hepatitis C/D/G  
111 virus, human immunodeficiency virus, hepatocellular carcinoma, alcoholic cirrhosis,

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3 112 schistosomiasis cirrhosis and any autoimmune liver disease were excluded.  
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5 114 ***Ethics statement***

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7 115 This study was approved by the Ethics Committee of the First Affiliated Hospital of  
8 116 the Medical College at Zhejiang University in China and was performed in  
9 117 accordance with the Helsinki Declaration.  
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11 119 ***Laboratory assessment***

12 120 All venous blood samples were obtained in the morning following a 12 h fast, within  
13 121 24 h after admission. All study participants were subjected to the following  
14 122 determinations: serum total protein (TP), albumin (ALB), total bilirubin (TB), alanine  
15 123 aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), total  
16 124 cholesterol (Tch), creatinine (Cr), prothrombin time (PT), complete blood cell counts,  
17 125 LMR in peripheral blood), international normalized ratio (INR) and the model for  
18 126 end-stage liver disease (MELD) score based on TB, Cr, INR and PT<sup>18</sup>. Complete  
19 127 blood cell counts were determined using a Sysmex XE-2100 automated hematology  
20 128 analyzer (Sysmex Corp, Kobe, Japan) with Sysmex reagents.  
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22 130 ***Statistical analysis***

23 131 Statistical analysis was performed using SPSS16.0 (SPSS Inc. IL, USA). Data were  
24 132 presented as mean  $\pm$  SD, median (range) or categorical data as percentages, if  
25 133 appropriate. The differences between two groups were assessed with an independent  
26 134 sample t-test, the Mann-Whitney U test or chi-square test, if appropriate. Multiple  
27 135 comparisons were performed by one-way analysis of variance (ANOVA) or  
28 136 Kruskal-Wallis H tests, if appropriate. Spearman correlation test was used in  
29 137 correlation analyses. The receiver operating characteristic (ROC) curve and cutoff  
30 138 values of LMR were obtained, and area under ROC curve (AUROC) was calculated  
31 139 to identify the best LMR and/or MELD score for predicting mortality in LC patients.  
32 140 These parameters were selected by stepwise regression, and multivariate logistic  
33 141 regression analyses were used to evaluate if low LMR was an independent factor for  
34 142 predicting mortality in LC patients by unadjusted model and adjusting for gender, TP,  
35 143 ALB, TB and MELD score. The high LMR group was used as the reference category.  
36 144 Statistical significance was defined at  $P < 0.05$ .  
37 145

38 146 **Results**

39 147  
40 148 ***Patients characteristics***

41 149 There were 479 LC patients, 134 CHB patients, and 240 healthy controls enrolled in  
42 150 our retrospective cohort. The patient characteristics are listed in Table 1. No statistical  
43 151 differences were observed for gender and age between the three groups. Whereas, TP,  
44 152 ALB, TB, ALT, AST, TG, Tch, Cr, INR, LMR, and WBC count were statistically  
45 153 different (all  $P < 0.05$ ). The MELD score and mortality of LC group were statistically  
46 154 higher than those of the CHB group ( $P < 0.001$ ).  
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156 ***LMR is lower in LC, especially in the non-surviving group***

157 The LMR was significantly lower in the LC group as compared to the control group  
158 (2.77 vs. 5.30, respectively) and to the CHB group (2.77 vs. 3.64;  $P < 0.01$ ). The  
159 non-surviving group exhibited a lower LMR (1.14) and higher MELD score (17.27)  
160 than the surviving group (3.11 and 7.99, respectively;  $P < 0.001$ ) (Fig. 1).

161  
162 ***LMR is closely correlated to the MELD score***

163 The LMR in LC group correlated with INR, ALB, Cr, TB, Tch, TG, TP, WBC and  
164 MELD score ( $r = -0.130, 0.127, -0.163, -0.211, 0.233, 0.173, 0.219, -0.288,$  and  
165  $-0.241$ ; all  $P < 0.05$ ). In non-surviving LC patients, LMR negatively correlated with  
166 MELD score with a higher correlation coefficient ( $r = -0.354$ ;  $P = 0.13$ ) compared  
167 with other indexes. In contrast, in the CHB group, LMR correlations with INR, ALB,  
168 TB, WBC, and MELD score were lower ( $r = -0.266, 0.249, -0.324, -0.186$  and  $-0.266$ ,  
169 respectively; all  $P < 0.05$ ), and this was even more pronounced for the control group  
170 where correlations with ALB and TP were only 0.198 and 0.142, respectively ( $P <$   
171  $0.05$ ).

172  
173 ***Combining LMR and MELD score increases the power for predicting mortality***

174 The ROC curve analyses were applied to estimate LMR and MELD score to predict  
175 mortality of LC patients (Fig. 2). LMR was changed into 1/LMR by inverse  
176 transformation. The AUROCs of 1/LMR and MELD score were 0.789 (95%  
177 confidence interval (CI): 0.735-0.842;  $P < 0.001$ ) and 0.878 (95% CI: 0.831-0.924;  $P$   
178  $< 0.001$ ), respectively. When 1/LMR and MELD score were combined, the AUC was  
179 0.885 (95% CI: 0.842-0.928;  $P < 0.001$ ). The cutoff values, sensitivity and specificity  
180 of MELD were 16.89%, 72.8% and 91.5%. For LMR the values were 2.10%, 77.2%,  
181 and 71.8%. When LMR was combined with MELD, the specificity reached up to  
182 97.4%.

183 The non-surviving patients had a higher level of WBC (6.75 [0.8-24.9] vs. 3.6  
184 [0.9-32.8] $\times 10^9/L$ ;  $P < 0.001$ ) and monocytes (0.73 [0.04-3.16] vs. 0.33  
185 [0.05-2.0] $\times 10^9/L$ ;  $P < 0.001$ ) than the surviving patients. Although the median and  
186 range of lymphocyte count of the non-surviving group were slightly lower than those  
187 of the surviving group (0.9 [0.1-4.3] vs. 1.00 [0.10-5.40] $\times 10^9/L$ ), the difference did  
188 not reach statistical significance ( $P = 0.166$ ). These data indicated that the lower LMR  
189 in the death group was mainly due to an increased number of monocytes and  
190 secondarily due to decreased lymphocytes.

191 To further explore the association of LMR and mortality, the 479 LC patients were  
192 divided into two groups according to the cutoff value (low LMR:  $LMR \leq 2.1$  and high  
193 LMR:  $LMR > 2.1$ ). The clinical characteristics and differences in variables between  
194 these groups are presented in Table 2. Patients with low LMR values had higher  
195 mortality, MELD score, TB, ALT, AST, Cr, INR and WBC, and had lower TP, ALB  
196 and Tch, compared with the high LMR subgroup.

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198 ***LMR is an independent prognostic factor of mortality in multivariate analysis***

199 Gender, MELD, low LMR (with high LMR as reference), TP, TB and ALB were

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3 200 selected by stepwise regression from the above parameters ( $P = 0.025, < 0.001, 0.048,$   
4 201  $0.006, < 0.001$  and  $0.021,$  respectively) with forward selection. Subsequent  
5 202 multivariate logistic regression analysis showed that low LMR levels were  
6 203 independent factors for predicting mortality in LC patients (Table 3).  
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### 9 205 **Discussion**

10 206 In the present retrospective study of HBV-LC a significant negative association was  
11 207 found between LMR in the peripheral blood and the MELD score. LMR of LC  
12 208 patients was statistically lower, and the MELD score and mortality of LC patients  
13 209 were statistically higher than those of CHB and control groups, especially in the  
14 210 non-surviving LC subgroup. Moreover, low LMR was an independent predictive  
15 211 factor of mortality. These results provide the first evidence for an association between  
16 212 LMR and mortality in LC patients.

17 213 Bacterial infections are an important cause of morbidity and mortality in patients with  
18 214 LC due to an impaired immune function together with an increased passage of  
19 215 bacteria from the gut (bacterial translocation [BT])<sup>4,5,20</sup>. Once infection occurs, it may  
20 216 lead to SIRS, which can cause serious complications such as severe sepsis, renal  
21 217 dysfunction, encephalopathy, coagulopathy and multiple organ failure<sup>20</sup>. SIRS occurs  
22 218 more frequently in patients with advanced cirrhosis and portal hypertension, and is  
23 219 associated with severity of liver disease and increased risk of death in LC patients<sup>4,5</sup>.  
24 220 The mortality of LC patients with infection has been reported to be more than twice  
25 221 that of patients without infection<sup>20</sup>. Monocytes are central mediators of the immune  
26 222 response and play a crucial role in the pathogenesis of liver cirrhosis. Endotoxin leads  
27 223 to monocyte activation and promotes the release into the serum of proinflammatory  
28 224 cytokines such as interleukin-1 (IL-1), IL-6, tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), and  
29 225 interferon- $\gamma$ . This release is proportional to liver disease severity. These cytokines act  
30 226 in an autocrine and paracrine fashion and result in the recruitment of inflammatory  
31 227 effector cells, such as polymorphonuclear cells<sup>20-22</sup>. The subsequent activation of  
32 228 nitric oxide (NO) via the cytokine cascade leads to vasodilatation<sup>24</sup>. Endotoxin,  
33 229 cytokines and NO are key elements in the pathogenesis of circulatory abnormalities in  
34 230 liver cirrhosis with infection. Li *et al.*, found that monocytes in HBV-related LC  
35 231 patients positively correlated with the endotoxin level and cirrhosis severity based on  
36 232 the Child-pugh classification, indicating that the endotoxin-driven monocyte  
37 233 activation was an important factor of SIRS and multiple organ failure<sup>25</sup>. Lee *et al.*,  
38 234 found that LC patients with hepatocellular carcinoma had a high monocyte ratio and  
39 235 that a preoperative monocyte ratio  $> 7\%$  was an independent risk factor for survival  
40 236 after hepatic resection<sup>11</sup>. Immune paralysis, defined as decreased human leukocyte  
41 237 antigen-DR (HLA-DR) expression on monocytes and indicating immune dysfunction,  
42 238 was found in LC patients. HLA-DR expression is a direct marker of monocyte  
43 239 function and a protective immune response in LC patients<sup>23</sup>. Monocyte HLA-DR  
44 240 expression is significantly reduced in those patients and falls in proportion to cirrhosis  
45 241 severity<sup>26,27</sup>. Therefore, LC patients may have high monocyte count but low  
46 242 monocyte HLA-DR expression for systemic inflammatory response and immune  
47 243 paralysis. Early diagnosis and treatment of infections can significantly reduce



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3 244 morbidity and improve survival of LC patients<sup>4,5,21,23,24,28</sup>.  
4 245 Inflammatory stimuli mainly affect the numbers of monocytes in the peripheral blood  
5 246 in LC patients, which contributes to LMR changes. In addition, the present study  
6 247 showed that lymphocytes in the death group showed a trend towards lower levels as  
7 248 compared with the survival group, without reaching statistical significance. Such a  
8 249 decline might be attributed to lymphocytopenia<sup>29,30</sup>. This is in accordance with  
9 250 Leithead *et al.*, who found that a lower lymphocyte count was associated with  
10 251 mortality in patients with end-stage cirrhosis listed for liver transplantation<sup>29</sup>.  
11 252 Lombardo *et al.*, also found that the progressive and severity-related decrease in  
12 253 peripheral blood T-lymphocyte suggested a progressive impairment of protective  
13 254 immune function in LC<sup>30</sup>. Therefore, high monocytes together with low lymphocytes  
14 255 may reflect the severity and progression of liver injury in LC patients.

15 256 LMR has been shown to be associated with tuberculosis and influenza virus infection  
16 257<sup>16,31</sup>. Recently, LMR has also been reported to predict survival and prognosis in  
17 258 various patient populations with malignant diseases<sup>12-15</sup>, and a decreased LMR has  
18 259 been shown to be significantly associated with a high risk for critical limb ischemia in  
19 260 peripheral arterial occlusive disease patients<sup>32</sup>. Compared with another novel  
20 261 inflammation index, the ability of NLR for predicting mortality (AUROC) in LC  
21 262 patients<sup>33</sup> was similar to LMR in our study. LMR was associated, in our study, with  
22 263 MELD score and was an independent predictive factor of mortality. Combined with  
23 264 the MELD score, the specificity for predicting mortality was improved. Additionally,  
24 265 the LMR is an easily available and low price biomarker. However, it should be noted  
25 266 that this was a retrospective study so that validation cohorts are warranted in order to  
26 267 confirm the present data. Moreover, these findings may only apply to HBV-related LC  
27 268 patients and, therefore, need to be validated in other etiologies of liver cirrhosis by  
28 269 future prospective clinical trials.

29 270 **Contributors** L.F. designed the experiments. G. F., J.W.Z. J.Z performed the  
30 271 experiments. Y.Z. and J.Y. analysed and interpreted all the data. J.Z and Y.Z. wrote  
31 272 the main manuscript text. All authors reviewed the manuscript.

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35 276 **Competing interests** None.

36 277 **Patient consent** Obtained.

37 278 **Ethics approval** This study was approved by the ethics committee of the First  
38 279 Affiliated Hospital of Zhejiang University School of Medicine, China.

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## Figure Legends

**Figure 1:** The boxplots of MELD score and LMR between surviving and non-surviving LC patients

LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score.

**Figure 2:** Receiver operating characteristic (ROC) curve analysis for predicting mortality by LMR and MELD score

LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score; 1/LMR+MELD, 1/LMR combined with MELD.

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**Table 1.** Basic characteristics of enrolled participants.

Variables	Control (240)	CHB (134)	LC (479)	<i>P</i> value
Female/male	61/179	34/100	126/353	0.956
Age (year)	50.6±9.69	48.9±8.04	50.8±10.8	0.163
HBsAg positive (yes/no)	0/240	134/0	479/0	-
HBeAg positive (yes/no)	0/240	66/68	184/295	0.024 <sup>#</sup>
TP (g/L)	71.6±3.79	67.3±6.83*	62.9±8.48* <sup>#</sup>	<0.001
ALB (g/L)	46.2±3.17	37.4±5.95*	33.2±5.61* <sup>#</sup>	<0.001
TBIL (μmol/L)	12(6-49)	21.5(5-309)*	31(5-839)* <sup>#</sup>	<0.001
ALT (U/L)	17(7-48)	61(9-1838)*	29(4-1882)* <sup>#</sup>	<0.001
AST (U/L)	19(12-46)	48(16-1235)*	40(8-4094)* <sup>#</sup>	<0.001
TG (mmol/L)	1.08(0.41-1.70)	1.33(0.44-4.14)*	0.79(0.3-3.59)* <sup>#</sup>	<0.001
Tch (mmol/L)	4.66(2.40-5.86)	4.04(1.6-8.17)*	2.89(0.74-9.73)*	<0.001
Cr (μmol/L)	73(39-100)	65(29-154)*	66(30-729)*	0.002
INR	0.94±0.05	1.21±0.23*	1.55±0.78* <sup>#</sup>	<0.001
WBC (10 <sup>12</sup> /L)	5.6(4.0-9.4)	4.75(2-12)*	3.9(0.8-32.8)* <sup>#</sup>	<0.001
LMR	5.30(1.4-13.2)	3.64(0.65-9.61)*	2.77(0.27-18.25)* <sup>#</sup>	<0.001
MELD score	-	5.89(0-23.63)	9.89(0.0-57.17)	<0.001 <sup>#</sup>
Mortality (yes/no)	-	1/133	92/387	<0.001 <sup>#</sup>

Data were presented as mean ± SD and median (range). CHB, chronic hepatitis B; LC, liver cirrhosis; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; TP, total protein; ALB, Albumin; TB, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; Tch, total cholesterol; Cr, creatinine; INR, international normalized ratio; WBC, white blood cell; LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score. *P*-value: Comparison among these three groups. <sup>#</sup>: LC group vs. CHB group. \*: *P* < 0.05 vs. the Control group

**Table 2.** Clinical characteristics of LC patients according to LMR cutoff value.

Variables	Low LMR (LMR $\leq$ 2.10, n=180)	High LMR (LMR $>$ 2.10, n=299)	<i>P</i> value
Female/male	52/128	74/225	0.319
Age (year)	51.5 $\pm$ 10.1	50.4 $\pm$ 11.2	0.290
TP (g/L)	60.6 $\pm$ 8.97	64.4 $\pm$ 7.87	<0.001
ALB (g/L)	32.3 $\pm$ 5.88	33.7 $\pm$ 5.40	0.009
TBIL ( $\mu$ mol/L)	50.5(8-839)	26(5-567)	<0.001
ALT (U/L)	31(5-1882)	28(4-1141)	0.468
AST (U/L)	44(16-4094)	37(8-1078)	<0.001
TG (mmol/L)	0.78(0.32-2.15)	0.79(0.30-3.59)	0.229
Tch (mmol/L)	2.37(0.79-5.29)	3.07(0.74-9.73)	<0.001
Cr ( $\mu$ mol/L)	72(30-729)	65(30-426)	<0.001
INR	1.82 $\pm$ 1.17	1.39 $\pm$ 0.29	<0.001
WBC ( $10^{12}$ /L)	5.20(0.8-32.8)	3.5(0.8-12.1)	<0.001
MELD score	14.52(0-57.2)	8.20(0.0-41.25)	<0.001
Mortality (yes/no)	71/109	21/278	<0.001

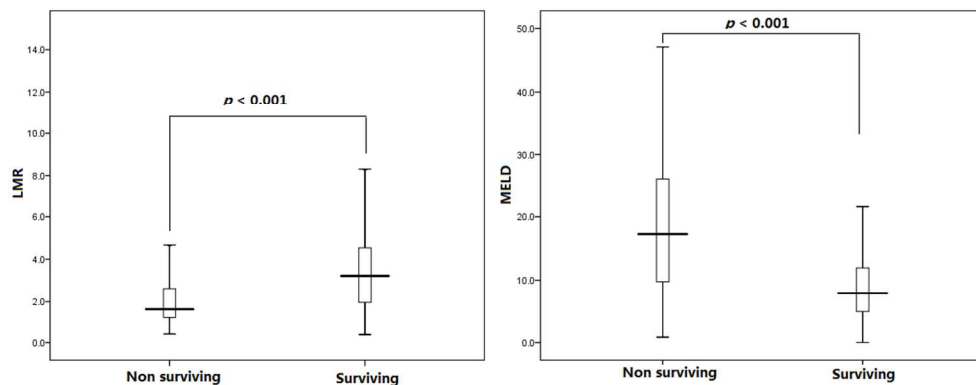
Data were presented as mean  $\pm$  SD and median (range). LMR, lymphocyte to monocyte ratio; LC, liver cirrhosis; TP, total protein; ALB, Albumin; TB, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; Tch, total cholesterol; Cr, creatinine; INR, international normalized ratio; WBC, white blood cell; MELD score, model for end-stage liver disease score.

**Table 3.** Odds ratios of low LMR for predicting mortality in LC patients

Models	Odds Ratio (95% CI)	<i>P</i> value
Model 1	8.623 (5.051-14.721)	< 0.001
Model 2	8.565 (5.013-14.634)	< 0.001
Model 3	3.392 (1.724-6.670)	< 0.001
Model 4	2.347 (1.134-4.859)	0.022

Odds ratios of low LMR were determined using high LMR as reference; model 1: unadjusted; model 2: adjusted for gender; model 3: adjusted for gender, TP, ALB, and TB; model 4: adjusted for gender, TP, ALB, TB and MELD score.



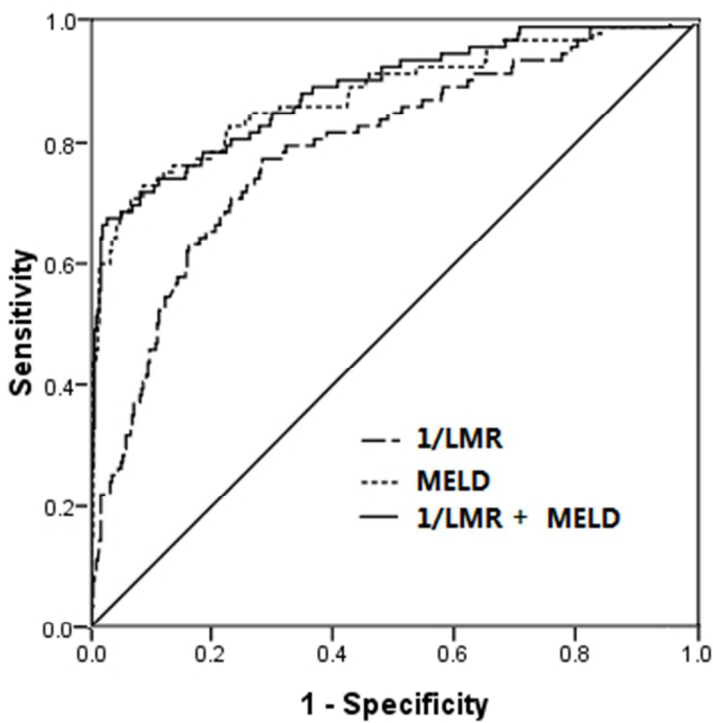


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**STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology\***  
**Checklist for cohort, case-control, and cross-sectional studies (combined)**

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	4-5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	no
Study size	10	Explain how the study size was arrived at	no
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	5
		(b) Describe any methods used to examine subgroups and interactions	5
		(c) Explain how missing data were addressed	no
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	5

		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	5
<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5,table 1
		(b) Give reasons for non-participation at each stage	no
		(c) Consider use of a flow diagram	no
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5,table 1
		(b) Indicate number of participants with missing data for each variable of interest	no
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	no
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	5-6,table 1-2
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6,table 3
		(b) Report category boundaries when continuous variables were categorized	6
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	6-7,table 3
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7-8
Generalisability	21	Discuss the generalisability (external validity) of the study results	7-8
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	8

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).

# BMJ Open

## Association between lymphocyte to monocyte ratio (LMR) and the mortality of liver cirrhosis: A retrospective cohort study

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<b>Primary Subject Heading</b>:	Gastroenterology and hepatology
Secondary Subject Heading:	Diagnostics, Infectious diseases
Keywords:	INFECTIOUS DISEASES, Gastrointestinal infections < GASTROENTEROLOGY, Hepatology < INTERNAL MEDICINE

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Manuscripts

1           **Association between lymphocyte to monocyte ratio (LMR) and the mortality of**  
2           **liver cirrhosis: A retrospective cohort study**

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8           Jie Zhang<sup>1</sup>, Guofang Feng<sup>2</sup>, Ying Zhao<sup>1</sup>, Juanwen Zhang<sup>1</sup>, Limin Feng<sup>1\*</sup>, Jing Yang

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## Abstract

**Objective:** Infection with hepatitis B virus (HBV) remains a major cause of liver cirrhosis (LC) in China. Recent reports suggest that the lymphocyte to monocyte ratio (LMR) is a potential biomarker for predicting clinical outcomes. In our study, we investigated if LMR can be used as a prognostic marker of mortality in LC patients.

**Design:** A retrospective cohort study.

**Setting:** HBV-infected patients with LC and patients with chronic hepatitis B infection (CHB) from the Department of Infectious Disease were enrolled and 240 healthy individuals were recruited from the healthcare center at the First Affiliated Hospital of Zhejiang University.

**Participants:** 479 HBV-infected patients with LC, 134 patients with CHB, and 240 healthy individuals were enrolled.

**Primary and secondary outcome measures:** The receiver operating characteristic (ROC) curve and multivariable logistic regression analysis after adjusting gender, total protein, albumin, total bilirubin and the model for end-stage liver disease (MELD) score, were used to evaluate the power of LMR for predicting 1-year mortality in LC patients.

**Results:** The LMR was statistically lower in LC patients. The MELD score and mortality were statistically higher in LC patients compared to those with chronic hepatitis B (CHB) and control groups. The area under the ROC curve (AUROC), cutoff values, sensitivity, and specificity of LMR for predicting mortality LC in the training cohort were 0.817 (95% confidence interval (CI): 0.746 - 0.888;  $P < 0.001$ ), 2.10, 82.6, and 78.8%, and these data were confirmed in the validation cohort. The multivariate logistic regression analysis showed that LMR was an independent predictive factor of mortality in LC (odds ratios [OR]: 2.347, 95% CI: [1.134 - 4.859];  $P = 0.022$ ).

**Conclusion:** Our results strongly suggest that low LMR can be considered as an independent biomarker for predicting mortality in patients with LC.

**Keywords:** liver cirrhosis; lymphocyte to monocyte ratio; the model for end-stage liver disease score

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60 **Strengths and limitations of this study**

- 61 ▪ LMR was lower in the LC group, especially in the non-surviving group, compared to
- 62 the control group and the CHB group.
- 63 ▪ LMR was closely correlated to the MELD score.
- 64 ▪ LMR was an easy parameter to achieve and the power for predicting mortality of
- 65 LMR was similar to that of MELD.
- 66 ▪ Low LMR levels were independent factors for predicting mortality in LC patients.
- 67 ▪ This was a retrospective study.

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## 71 Introduction

72 Liver cirrhosis (LC) is a common hepatic disease in China, and represents an  
73 increasing cause of morbidity and mortality<sup>1,2</sup>. Hepatitis B virus (HBV) infection  
74 remains a major cause of LC in China, with a 3% yearly incidence of decompensated  
75 cirrhosis<sup>3</sup>. Systemic inflammatory response syndrome (SIRS) is relatively common in  
76 patients with complicated cirrhosis<sup>4,5</sup>. SIRS can further deteriorate liver function,  
77 maximize the risk of complications and increase the mortality rate of LC patients<sup>4,5</sup>.  
78 SIRS is usually measured by peripheral blood count-based parameters, such as  
79 neutrophils, lymphocytes, monocytes, red blood cell distribution width (RDW), mean  
80 platelet volume (MPV) or platelet count. These parameters have been reported to be  
81 independent predictive markers of clinical outcome in cancer and different states of  
82 HBV-related hepatic disorders<sup>6-10</sup>. Among these inflammatory parameters, the  
83 neutrophil-lymphocyte ratio (NLR), RDW and monocyte ratio have been proposed as  
84 easily accessible and reliable markers<sup>6-8,11</sup>. Several recent studies suggest that the  
85 lymphocyte to monocyte ratio (LMR) is a cheap, readily available and reproducible  
86 test with potential for predicting clinical outcomes of patients with solid tumors and  
87 hematologic malignancy, including nasopharyngeal carcinoma, colorectal cancer,  
88 pancreatic cancer, and lymphoma<sup>12-15</sup>. Moreover, Merakoulias *et al.*, found that, in  
89 90% of patients who had influenza virus, lymphopenia and/or monocytosis, LMR  
90 could be used as a time-saving and cost-effective screening test for influenza virus  
91 infection, leading to early antiviral treatment and a decreased incidence of  
92 complications<sup>16</sup>. Assuming that there may be association between LMR and LC  
93 severity, we investigated the potential prognostic value of LMR as a biomarker in  
94 HBV-related LC.

95 To the best of our knowledge, there is no data on LMR as a LC diagnostic measure.  
96 We therefore performed a retrospective cohort study to investigate, the association  
97 between LMR in peripheral blood in LC patients, with special emphasis on the value  
98 of LMR for predicting the mortality of LC patients.

## 100 Subjects and Methods

### 102 Subjects

103 We continuously analyzed all 547 patients with HBV-related liver cirrhosis (LC) from  
104 the Department of Infectious Disease, The First Affiliated Hospital, School of  
105 Medicine, Zhejiang University, between October 2012 and October 2013. Sixty-eight  
106 LC patients with a concurrent infection of hepatitis C/D/E/G virus (n = 3), human  
107 immunodeficiency virus (HIV, n = 1), hepatocellular carcinoma (HCC, n = 56),  
108 alcoholic cirrhosis (n = 5), schistosomiasis cirrhosis (n = 1), and any autoimmune  
109 liver disease (n = 2) were excluded. The remaining 479 LC patients were enrolled in  
110 our retrospective cohort study. All clinical data were retrieved from medical records at  
111 the Department of Infectious Disease. One hundred thirty-four patients with chronic  
112 hepatitis B infection (CHB), with no statistical differences in age and gender versus  
113 LC patients, were selected from the Department of Infectious Disease without a  
114 concurrent infection of hepatitis C/D/G virus, HIV, HCC, and any autoimmune liver

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3 115 disease. CHB and LC were diagnosed according to the criteria of the 2000 Xi'an viral  
4 116 hepatitis management scheme<sup>17</sup>. Liver cirrhosis was diagnosed based on the history  
5 117 of liver disease, clinical manifestations, laboratory tests, imaging tests, and, whenever  
6 118 feasible, liver biopsy<sup>17</sup>. CHB was defined as previous hepatitis B or hepatitis B  
7 119 surface antigen (HBsAg) positivity for > 6 months and persistently positive HBsAg  
8 120 and/or HBV DNA<sup>17-18</sup>. The LC group was subdivided into two subgroups according to  
9 121 mortality at 1-year of follow up. For LC and CHB patients discharged from hospital,  
10 122 1-year prognostic information was obtained by checking medical records or by  
11 123 contacting the patients' family members. One hundred and eight LC patients were  
12 124 decompensated. Out of 92 LC patients mainly died of upper gastrointestinal bleeding  
13 125 (n = 40), hepatic encephalopathy (n = 28), hepatorenal syndrome (n = 15), infection (n  
14 126 = 5), or of other causes (n = 4). Two hundred and forty healthy controls with no  
15 127 statistical differences in age and gender versus LC patients were selected from health  
16 128 examination population who underwent a general health checkup that included a  
17 129 physical examination and some clinical laboratory tests at the Health Care Centre of  
18 130 the First Affiliated Hospital of Medical College of Zhejiang University. They  
19 131 corresponded to HBsAg negative individuals with normal liver function, normal renal  
20 132 function, and no infection. One hundred and thirty-four CHB patients and 240 healthy  
21 133 controls were used to compare basic characteristics with 479 LC patients.  
22 134

### 23 135 ***Ethics statement***

24 136 This study was approved by the Ethics Committee of the First Affiliated Hospital of  
25 137 the Medical College at Zhejiang University in China and was performed in  
26 138 accordance with the Helsinki Declaration.  
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### 28 140 ***Laboratory assessment***

29 141 All venous blood samples were obtained in the morning following a 12 h fast, within  
30 142 24 h after admission. All study participants were subjected to the following  
31 143 determinations: serum total protein (TP), albumin (ALB), total bilirubin (TB), alanine  
32 144 aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), total  
33 145 cholesterol (Tch), creatinine (Cr), prothrombin time (PT), complete blood cell counts,  
34 146 LMR in peripheral blood), international normalized ratio (INR) and the model for  
35 147 end-stage liver disease (MELD) score based on TB, Cr, INR and PT<sup>18</sup>. Complete  
36 148 blood cell counts were determined using a Sysmex XE-2100 automated hematology  
37 149 analyzer (Sysmex Corp, Kobe, Japan) with Sysmex reagents.  
38 150

### 39 151 ***Statistical analysis***

40 152 Statistical analysis was performed using SPSS16.0 (SPSS Inc. IL, USA). Data were  
41 153 presented as mean  $\pm$  SD, median (range) or categorical data as percentages, if  
42 154 appropriate. The differences between two groups were assessed with an independent  
43 155 sample t-test, the Mann-Whitney U test or chi-square test, if appropriate. Multiple  
44 156 comparisons were performed by one-way analysis of variance (ANOVA) or  
45 157 Kruskal-Wallis H tests, if appropriate. Spearman correlation test was used in  
46 158 correlation analyses. The receiver operating characteristic (ROC) curve and cutoff  
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159 values of LMR were obtained, and area under ROC curve (AUROC) was calculated  
160 to identify the best LMR and/or MELD score for predicting mortality in LC patients.  
161 For AUROC analysis of combined 1/LMR and MELD score for predicting mortality  
162 in LC patients, predictive models of 1/LMR, MELD, and 1/LMR + MELD were first  
163 developed by binary logistic regression analyses, respectively. Probabilities of 1/LMR,  
164 MELD, and 1/LMR + MELD were then generated, respectively, and used as three  
165 new input variables for the ROC curve analysis (shown in Figure 2). These  
166 parameters were selected by stepwise regression, and multivariate logistic regression  
167 analyses were used to evaluate if low LMR was an independent factor for predicting  
168 mortality in LC patients by an unadjusted model and adjusting for gender, TP, ALB,  
169 TB and MELD score. The high LMR group was used as the reference category.  
170 Statistical significance was defined at  $P < 0.05$ .

## 171 Results

### 172 *Patient characteristics*

173  
174 There were 479 LC patients, 134 CHB patients, and 240 healthy controls enrolled in  
175 our study. The patient characteristics are listed in Table 1. No statistical differences  
176 were observed for gender and age between the three groups. Whereas, TP, ALB, TB,  
177 ALT, AST, TG, Tch, Cr, INR, LMR, and WBC count had statistically differences (all  
178  $P < 0.05$ ). The MELD score and mortality of the LC group were statistically higher  
179 than those of the CHB group ( $P < 0.001$ ).

### 180 *LMR is lower in LC, especially in the non-surviving group*

181  
182 The LMR was significantly lower in the LC group compared to the control group  
183 (2.77 vs. 5.30, respectively) and to the CHB group (2.77 vs. 3.64;  $P < 0.01$ ). The  
184 clinical characteristics and differences in variables between non-surviving and  
185 surviving LC patients are presented in Table 2. The non-surviving patients had lower  
186 LMR (Fig. 1), TP, ALB, and Tch, and higher MELD score, TB, ALT, AST, TG, Cr,  
187 INR, WBC, monocytes, and rate of decompensated cirrhosis, compared with  
188 surviving patients. The median and range of lymphocyte count of the non-surviving  
189 group were slightly lower than those of the surviving group, but the difference did not  
190 reach statistical significance. These data indicate that the lower LMR in the  
191 non-surviving group was mainly due to an increased number of monocytes and  
192 secondarily due to decreased lymphocytes. LMR resulted in no significant differences  
193 in LC patients whose primary cause of death was upper gastrointestinal bleeding,  
194 hepatic encephalopathy, or hepatorenal syndrome ((1.35[0.35-17.75]),  
195 1.42[0.27-18.20], 1.39[0.39-18.25],  $p=0.955$ ).

### 196 *LMR is closely correlated to the MELD score*

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198 The LMR in the LC group negatively correlated with MELD score ( $r = -0.241$ ;  $P <$   
199  $0.05$ ), especially in non-surviving LC patients, LMR negatively correlated with  
200 MELD score with a higher correlation coefficient ( $r = -0.354$ ;  $P = 0.013$ ) compared  
201 with LMR in surviving LC patients.  
202

203

***The power for predicting 1-year mortality of LMR***

204 The enrolled 479 LC patients were divided into two cohorts: the training cohort (n =  
205 239) and the validation cohort (n = 240). The ROC curve analyses of the training  
206 cohort were applied to estimate LMR and MELD score to predict mortality of LC  
207 patients (Fig. 2). LMR was changed into 1/LMR by inverse transformation. The  
208 AUROCs of 1/LMR and MELD score were 0.817 (95% confidence interval (CI):  
209 0.746 - 0.888;  $P < 0.001$ ) and 0.868 (95% CI: 0.795 - 0.941;  $P < 0.001$ ), respectively.  
210 The cutoff values, sensitivity and specificity of MELD were 19.1, 73.9 and 96.4%.  
211 LMR values were 2.10, 82.6, and 78.8%. When 1/LMR and MELD score were  
212 combined, the AUC was 0.876 (95% CI: 0.808 - 0.945;  $P < 0.001$ ), only slightly  
213 higher than AUC of MELD score, and neither the specificity (71.7%) nor the  
214 sensitivity (96.9%) was significantly improved. Applying the LMR to the validation  
215 cohort, the AUROCs of 1/LMR, MELD score, and 1/LMR+MELD were 0.773 (95%  
216 CI: 0.692 - 0.854;  $P < 0.001$ ), 0.887 (95% CI: 0.829 - 0.945;  $P < 0.001$ ), 0.890 (95%  
217 CI: 0.836 - 0.944;  $P < 0.001$ ), respectively. There were no significant differences in  
218 the AUCs of LMR between the estimation and validation cohorts ( $Z = 0.741$ ,  $P =$   
219 0.053). To summarize, LMR was an easy parameter to achieve and the power for  
220 predicting mortality of LMR was similar to that of MELD.  
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222

***LMR is an independent prognostic factor of mortality in multivariate analysis***

223 Gender, MELD, low LMR (LMR  $\leq 2.10$ , with high LMR  $> 2.10$  as a reference), TP,  
224 TB and ALB were selected by stepwise regression from the above parameters ( $P =$   
225 0.025,  $< 0.001$ , 0.048, 0.006,  $< 0.001$  and 0.021, respectively) with forward selection.  
226 Subsequent multivariate logistic regression analysis showed that low LMR was an  
227 independent factor for predicting mortality in LC patients (Table 3).  
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**Discussion**

230 In the present retrospective study of HBV-LC a significant negative association was  
231 found between LMR in the peripheral blood and the MELD score. LMR of LC  
232 patients was statistically lower, and the MELD score and mortality of LC patients  
233 were statistically higher than those of CHB and control groups, especially in the  
234 non-surviving LC subgroup. Moreover, low LMR was an independent predictive  
235 factor of mortality. These results provide the first evidence for an association between  
236 LMR and mortality in LC patients.  
237

238 Bacterial infections are an important cause of morbidity and mortality in patients with  
239 LC due to an impaired immune function together with an increased passage of  
240 bacteria from the gut (bacterial translocation [BT])<sup>4,5,19</sup>. Once infection occurs, it may  
241 lead to SIRS, which can cause serious complications such as severe sepsis, renal  
242 dysfunction, encephalopathy, coagulopathy and multiple organ failure<sup>19</sup>. SIRS occurs  
243 more frequently in patients with advanced cirrhosis and portal hypertension, and is  
244 associated with severity of liver disease and increased risk of death in LC patients<sup>4,5</sup>.  
245 The mortality of LC patients with infection has been reported to be more than twice  
246 that of patients without infection<sup>19</sup>. Monocytes are central mediators of the immune

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3 247 response and play a crucial role in the pathogenesis of liver cirrhosis. Endotoxin leads  
4 248 to monocyte activation and promotes the release of proinflammatory cytokines such  
5 249 as interleukin-1 (IL-1), IL-6, tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), and interferon- $\gamma$  into the  
6 250 serum. This release is proportional to liver disease severity. These cytokines act in an  
7 251 autocrine and paracrine fashion and result in the recruitment of inflammatory effector  
8 252 cells, such as polymorphonuclear cells<sup>19-21</sup>. The subsequent activation of nitric oxide  
9 253 (NO) *via* the cytokine cascade leads to vasodilatation<sup>22</sup>. Endotoxin, cytokines and NO  
10 254 are key elements in the pathogenesis of circulatory abnormalities in liver cirrhosis  
11 255 with infection. Li *et al.*, found that monocytes in HBV-related LC patients positively  
12 256 correlated with the endotoxin level and cirrhosis severity based on the Child-pugh  
13 257 classification, indicating that the endotoxin-driven monocyte activation was an  
14 258 important factor of SIRS and multiple organ failure<sup>23</sup>. Lee *et al.*, found that LC  
15 259 patients with hepatocellular carcinoma had a high monocyte ratio and that a  
16 260 preoperative monocyte ratio > 7% was an independent risk factor for survival after  
17 261 hepatic resection<sup>11</sup>. Immune paralysis, defined as decreased human leukocyte  
18 262 antigen-DR (HLA-DR) expression on monocytes and indicating immune dysfunction,  
19 263 was found in LC patients. HLA-DR expression is a direct marker of monocyte  
20 264 function and a protective immune response in LC patients<sup>24</sup>. Monocyte HLA-DR  
21 265 expression is significantly reduced in those patients and falls in proportion to cirrhosis  
22 266 severity<sup>25,26</sup>. Therefore, LC patients may have high monocyte count but low  
23 267 monocyte HLA-DR expression for systemic inflammatory response and immune  
24 268 paralysis. Early diagnosis and treatment of infections can significantly reduce  
25 269 morbidity and improve survival of LC patients<sup>4,5,20,22,23,27</sup>.

26 270 Inflammatory stimuli mainly affect the numbers of monocytes in the peripheral blood  
27 271 in LC patients, which contributes to LMR changes. In addition, the present study  
28 272 showed that lymphocytes in the non-survival group showed a trend towards lower  
29 273 levels as compared with the survival group, without reaching statistical significance.  
30 274 Such a decline might be attributed to lymphocytopenia<sup>28,29</sup>. This is in accordance  
31 275 with Leithead *et al.*, who found that a lower lymphocyte count was associated with  
32 276 mortality in patients with end-stage cirrhosis listed for liver transplantation<sup>26</sup>.  
33 277 Lombardo *et al.*, also found that the progressive and severity-related decrease in  
34 278 peripheral blood T-lymphocyte suggested a progressive impairment of protective  
35 279 immune function in LC<sup>29</sup>. Therefore, high monocytes together with low lymphocytes  
36 280 may reflect the severity and progression of liver injury in LC patients.

37 281 LMR has been shown to be associated with tuberculosis and influenza virus infection  
38 282<sup>16,30</sup>. Recently, LMR has also been reported to predict survival and prognosis in  
39 283 various patient populations with malignant diseases<sup>12-15</sup>, and a decreased LMR has  
40 284 been shown to be significantly associated with a high risk for critical limb ischemia in  
41 285 peripheral arterial occlusive disease patients<sup>31</sup>. Compared with another novel  
42 286 inflammation index, the ability of NLR for predicting mortality (AUROC) in LC  
43 287 patients<sup>32</sup> was similar to LMR in our study. LMR was associated, in our study, with  
44 288 MELD score, the power for predicting mortality of LMR was similar to that of MELD,  
45 289 and was an independent predictive factor of mortality. In addition, the LMR is an  
46 290 easily available and low price biomarker. However, it should be noted that this was a

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3 291 retrospective study so that prospective cohorts are warranted in order to confirm the  
4 292 present data. Moreover, these findings may only apply to HBV-related LC patients  
5 293 and, therefore, need to be validated in other etiologies of liver cirrhosis by future  
6  
7 294 prospective clinical trials.

8 295 **Contributorship statement** L.F. designed the experiments. G. F., JW.Z. J.Z  
9 296 performed the experiments. Y.Z. and J.Y. analyzed and interpreted all the data. J.Z and  
10 297 Y.Z. wrote the main manuscript text. All authors reviewed the manuscript.

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16 303 Ethics approval This study was approved by the ethics committee of the First  
17 304 Affiliated Hospital of Zhejiang University School of Medicine, China. Patient consent  
18 305 Obtained.

19 306 **Data sharing statement** No additional unpublished data are available.  
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## Figure Legends

**Figure 1:** The boxplots of MELD score and LMR between surviving and non-surviving LC patients

LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score.

**Figure 2:** Receiver operating characteristic (ROC) curve analysis for predicting mortality by LMR and MELD score in the training cohort.

LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score; 1/LMR+MELD, 1/LMR combined with MELD.

For peer review only

**Table 1.** Basic characteristics of enrolled participants.

Variables	Control (240)	CHB (134)	LC (479)	<i>P</i> value
Female/male	61/179	34/100	126/353	0.956
Age (year)	50.6±9.69	48.9±8.04	50.8±10.8	0.163
HBsAg positive (yes/no)	0/240	134/0	479/0	-
HBeAg positive (yes/no)	0/240	66/68	184/295	0.024 <sup>#</sup>
TP (g/L)	71.6±3.79	67.3±6.83*	62.9±8.48* <sup>#</sup>	<0.001
ALB (g/L)	46.2±3.17	37.4±5.95*	33.2±5.61* <sup>#</sup>	<0.001
TBIL (μmol/L)	12(6-49)	21.5(5-309)*	31(5-839)* <sup>#</sup>	<0.001
ALT (U/L)	17(7-48)	61(9-1838)*	29(4-1882)* <sup>#</sup>	<0.001
AST (U/L)	19(12-46)	48(16-1235)*	40(8-4094)* <sup>#</sup>	<0.001
TG (mmol/L)	1.08(0.41-1.70)	1.33(0.44-4.14)*	0.79(0.3-3.59)* <sup>#</sup>	<0.001
Tch (mmol/L)	4.66(2.40-5.86)	4.04(1.6-8.17)*	2.89(0.74-9.73)*	<0.001
Cr (μmol/L)	73(39-100)	65(29-154)*	66(30-729)*	0.002
INR	0.94±0.05	1.21±0.23*	1.55±0.78* <sup>#</sup>	<0.001
WBC (10 <sup>12</sup> /L)	5.6(4.0-9.4)	4.75(2-12)*	3.9(0.8-32.8)* <sup>#</sup>	<0.001
LMR	5.30(1.4-13.2)	3.64(0.65-9.61)*	2.77(0.27-18.25)* <sup>#</sup>	<0.001
MELD score	-	5.89(0-23.63)	9.89(0-57.17)	<0.001 <sup>#</sup>
Mortality (yes/no)	-	1/133	92/387	<0.001 <sup>#</sup>

Data were presented as mean ± SD and median (range). CHB, chronic hepatitis B; LC, liver cirrhosis; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; TP, total protein; ALB, Albumin; TB, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; Tch, total cholesterol; Cr, creatinine; INR, international normalized ratio; WBC, white blood cell; LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score. *P*-value: Comparison among these three groups. <sup>#</sup>: LC group vs. CHB group. \*: *P* < 0.05 vs. the Control group

**Table 2.** The clinical characteristics and differences in variables between non-surviving and surviving LC patients.

Variables	Non-surviving (n=92)	Surviving (n=387)	<i>P</i> value
Female/male	30/62	96/291	0.127
Age (year)	53.8±10.3	50.1±10.8	0.003
TP (g/L)	56.4±8.40	64.5±7.74	<0.001
ALB (g/L)	29.7±5.17	34.0±5.40	<0.001
TBIL (µmol/L)	292.5(9-839)	27(5-836)	<0.001
ALT (U/L)	48(4-1882)	27(5-475)	<0.001
AST (U/L)	66(10-4094)	37(8-440)	<0.001
TG (mmol/L)	0.88(0.30-2.15)	0.76(0.33-3.59)	0.022
Tch (mmol/L)	1.83(0.74-5.29)	3.02(0.94-9.73)	<0.001
Cr (µmol/L)	73.5(30-729)	65(30-326)	<0.001
INR	2.23±1.51	1.39±0.28	<0.001
WBC (10 <sup>9</sup> /L)	6.75(0.8-24.9)	3.6(0.9-32.8)	<0.001
Monocytes(10 <sup>9</sup> /L)	0.73 (0.04-3.16)	0.33 (0.05-2.0)	<0.001
Lymphocyte(10 <sup>9</sup> /L)	0.9 (0.1-4.3)	1.00 (0.10-5.40)	0.166
LMR	1.41(0.27-18.25)	3.10(0.38-14.58)	<0.001
MELD score	22.94(0.84-57.17)	8.49(0-35.33)	<0.001
Decompensated cirrhosis (yes/no)	82/10	26/361	<0.001

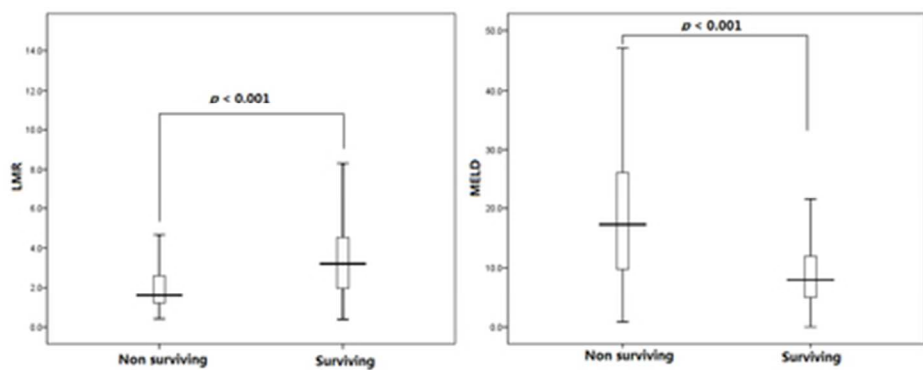
Data were presented as mean ± SD and median (range). LMR, lymphocyte to monocyte ratio; LC, liver cirrhosis; TP, total protein; ALB, Albumin; TB, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; Tch, total cholesterol; Cr, creatinine; INR, international normalized ratio; WBC, white blood cell; MELD score, model for end-stage liver disease score.

**Table 3.** Odds ratios of low LMR for predicting mortality in LC patients

Models	Odds Ratio (95% CI)	<i>P</i> value
Model 1	8.623 (5.051-14.721)	< 0.001
Model 2	8.565 (5.013-14.634)	< 0.001
Model 3	3.392 (1.724-6.670)	< 0.001
Model 4	2.347 (1.134-4.859)	0.022

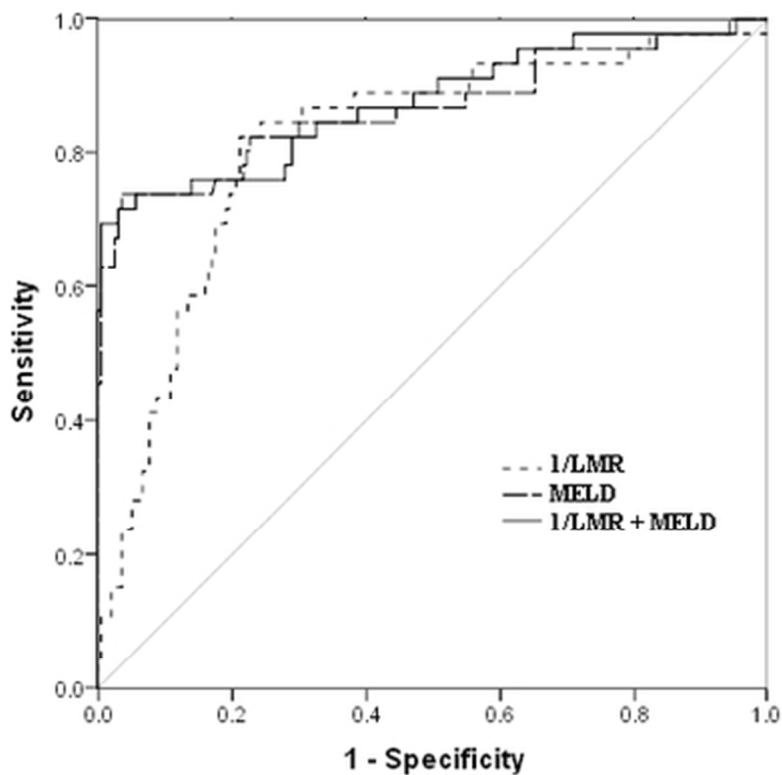
Odds ratios of low LMR were determined using high LMR as reference; model 1: unadjusted; model 2: adjusted for gender; model 3: adjusted for gender, TP, ALB, and TB; model 4: adjusted for gender, TP, ALB, TB and MELD score.

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**STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology\***  
**Checklist for cohort, case-control, and cross-sectional studies (combined)**

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	4-5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	no
Study size	10	Explain how the study size was arrived at	no
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	5-6
		(b) Describe any methods used to examine subgroups and interactions	5-6
		(c) Explain how missing data were addressed	no
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	5-6

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		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	5-6
<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5,table 1
		(b) Give reasons for non-participation at each stage	no
		(c) Consider use of a flow diagram	no
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5,table 1
		(b) Indicate number of participants with missing data for each variable of interest	no
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	no
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	5-6,table 1-2
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6,table 3
		(b) Report category boundaries when continuous variables were categorized	6
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	6-7,table 3
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8-9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7-8
Generalisability	21	Discuss the generalisability (external validity) of the study results	7-9
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).

# BMJ Open

## Association between lymphocyte to monocyte ratio (LMR) and the mortality of HBV-related liver cirrhosis: A retrospective cohort study

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<b>Primary Subject Heading</b>:	Gastroenterology and hepatology
Secondary Subject Heading:	Diagnostics, Infectious diseases
Keywords:	INFECTIOUS DISEASES, Epidemiology < INFECTIOUS DISEASES, Biochemistry < BASIC SCIENCES, Hepatobiliary disease < GASTROENTEROLOGY

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Manuscripts

1           **Association between lymphocyte to monocyte ratio (LMR) and the mortality of**  
2           **HBV-related liver cirrhosis: A retrospective cohort study**

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8           Jie Zhang<sup>1</sup>, Guofang Feng<sup>2</sup>, Ying Zhao<sup>1</sup>, Juanwen Zhang<sup>1</sup>, Limin Feng<sup>1\*</sup>, Jing Yang

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1  
2  
3 **Abstract**

4 **Objective:** Infection with hepatitis B virus (HBV) remains a major cause of liver  
5 cirrhosis (LC) in China. Recent reports suggest that the lymphocyte to monocyte ratio  
6 (LMR) is a potential biomarker for predicting clinical outcomes. In our study, we  
7 investigated if LMR can be used as a prognostic marker of mortality in HBV-related  
8 LC patients.

9 **Design:** A retrospective cohort study.

10 **Setting:** HBV-infected patients with LC and patients with chronic hepatitis B  
11 infection (CHB) from the Department of Infectious Disease were enrolled and 240  
12 healthy individuals were recruited from the healthcare center at the First Affiliated  
13 Hospital of Zhejiang University.

14 **Participants:** 479 HBV-infected patients with LC, 134 patients with CHB, and 240  
15 healthy individuals were enrolled.

16 **Primary and secondary outcome measures:** The receiver operating characteristic  
17 (ROC) curve and multivariable logistic regression analysis after adjusting total protein,  
18 albumin, total bilirubin and the model for end-stage liver disease (MELD) score, were  
19 used to evaluate the power of LMR for predicting 1-year mortality in LC patients.

20 **Results:** The LMR was statistically lower in LC patients. The MELD score and  
21 mortality were statistically higher in LC patients compared to those with chronic  
22 hepatitis B (CHB) and control groups. The area under the ROC curve (AUROC),  
23 cutoff values, sensitivity, and specificity of LMR for predicting mortality LC in the  
24 training cohort were 0.817 (95% confidence interval (CI): 0.746 - 0.888;  $P < 0.001$ ),  
25 2.10, 82.6, and 78.8%, and these data were confirmed in the validation cohort. The  
26 multivariate logistic regression analysis showed that LMR was an independent  
27 predictive factor of mortality in LC (odds ratios [OR]: 2.370, 95% CI: [1.070-5.249];  
28  $P = 0.033$ ).

29 **Conclusion:** Our results strongly suggest that low LMR can be considered as an  
30 independent biomarker for predicting mortality in patients with LC.

31 **Keywords:** liver cirrhosis; lymphocyte to monocyte ratio; the model for end-stage  
32 liver disease score  
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60 **Strengths and limitations of this study**

- 61 ▪ LMR was lower in the LC group, especially in the non-surviving group, compared to
- 62 the control group and the CHB group.
- 63 ▪ LMR was closely correlated to the MELD score.
- 64 ▪ LMR was an easy parameter to achieve and the power for predicting mortality of
- 65 LMR was similar to that of MELD.
- 66 ▪ Low LMR levels were independent factors for predicting mortality in LC patients.
- 67 ▪ This was a retrospective study.

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## 71 Introduction

72 Liver cirrhosis (LC) is a common hepatic disease in China, and represents an  
73 increasing cause of morbidity and mortality<sup>1,2</sup>. Hepatitis B virus (HBV) infection  
74 remains a major cause of LC in China, with a 3% yearly incidence of decompensated  
75 cirrhosis<sup>3</sup>. Systemic inflammatory response syndrome (SIRS) is relatively common in  
76 patients with complicated cirrhosis<sup>4,5</sup>. SIRS can further deteriorate liver function,  
77 maximize the risk of complications and increase the mortality rate of LC patients<sup>4,5</sup>.  
78 SIRS is usually measured by peripheral blood count-based parameters, such as  
79 neutrophils, lymphocytes, monocytes, red blood cell distribution width (RDW), mean  
80 platelet volume (MPV) or platelet count. These parameters have been reported to be  
81 independent predictive markers of clinical outcome in cancer and different states of  
82 HBV-related hepatic disorders<sup>6-10</sup>. Among these inflammatory parameters, the  
83 neutrophil-lymphocyte ratio (NLR), RDW and monocyte ratio have been proposed as  
84 easily accessible and reliable markers<sup>6-8,11</sup>. Several recent studies suggest that the  
85 lymphocyte to monocyte ratio (LMR) is a cheap, readily available and reproducible  
86 test with potential for predicting clinical outcomes of patients with solid tumors and  
87 hematologic malignancy, including nasopharyngeal carcinoma, colorectal cancer,  
88 pancreatic cancer, and lymphoma<sup>12-15</sup>. Moreover, Merkoulias *et al.*, found that, in 90%  
89 of patients who had influenza virus, lymphopenia and/or monocytosis, LMR could be  
90 used as a time-saving and cost-effective screening test for influenza virus infection,  
91 leading to early antiviral treatment and a decreased incidence of complications<sup>16</sup>.  
92 Assuming that there may be association between LMR and LC severity, we  
93 investigated the potential prognostic value of LMR as a biomarker in HBV-related  
94 LC.

95 To the best of our knowledge, there is no data on LMR as a LC diagnostic measure.  
96 We therefore performed a retrospective cohort study to investigate, the association  
97 between LMR in peripheral blood in LC patients, with special emphasis on the value  
98 of LMR for predicting the mortality of LC patients.

## 100 Subjects and Methods

### 102 Subjects

103 We continuously analyzed all 547 patients with HBV-related liver cirrhosis (LC) from  
104 the Department of Infectious Disease, The First Affiliated Hospital, School of  
105 Medicine, Zhejiang University, between October 2012 and October 2013. Sixty-eight  
106 LC patients with a concurrent infection of hepatitis C/D/E/G virus (n = 3), human  
107 immunodeficiency virus (HIV, n = 1), hepatocellular carcinoma (HCC, n = 56),  
108 alcoholic cirrhosis (n = 5), schistosomiasis cirrhosis (n = 1), and any autoimmune  
109 liver disease (n = 2) were excluded. The remaining 479 LC patients were enrolled in  
110 our retrospective cohort study. All clinical data were retrieved from medical records at  
111 the Department of Infectious Disease. One hundred thirty-four patients with chronic  
112 hepatitis B infection (CHB), with no statistical differences in age and gender versus  
113 LC patients, were selected from the Department of Infectious Disease without a  
114 concurrent infection of hepatitis C/D/G virus, HIV, HCC, and any autoimmune liver

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3 115 disease between October 2012 and October 2013. CHB and LC were diagnosed  
4 116 according to the criteria of the 2000 Xi'an viral hepatitis management scheme<sup>17</sup>.  
5 117 Liver cirrhosis was diagnosed based on the history of liver disease, clinical  
6 118 manifestations, laboratory tests, imaging tests, and, whenever feasible, liver biopsy<sup>17</sup>.  
7 119 Decompensated cirrhosis was defined by the presence of jaundice, ascites, variceal  
8 120 haemorrhage or hepatic encephalopathy<sup>17-18</sup>. The causes of admission in LC patients  
9 121 *without* decompensation were mainly jaundice, hypodynamia, and portal hypertension  
10 122 manifestations (esophageal varices, hypersplenism). The causes of admission in LC  
11 123 patients *with* decompensation were ascites, upper gastrointestinal bleeding  
12 124 (esophageal varices), hepatic encephalopathy, hepato-renal syndrome, and infection.  
13 125 CHB was defined as hepatitis B or hepatitis B surface antigen (HBsAg) positivity for >  
14 126 6 months, and persistently positive HBsAg and/or HBV DNA<sup>17-18</sup>. The LC group was  
15 127 subdivided into two subgroups according to mortality at 1-year of follow up. 227 LC  
16 128 and 33 CHB were under antiviral therapy before admission, 189 LC patients and 76  
17 129 CHB were under antiviral therapy after admission, altogether 416 LC (86.8%) and  
18 130 109 (81.3%) CHB were under antiviral therapy. For LC and CHB patients discharged  
19 131 from hospital, 1-year prognostic information was obtained by checking medical  
20 132 records or by contacting the patients' family members. One hundred and eight LC  
21 133 patients were decompensated. Out of 92 LC patients died of upper gastrointestinal  
22 134 bleeding (n = 40), hepatic encephalopathy (n = 28), hepato-renal syndrome (n = 15),  
23 135 infection (n = 5), electrolyte disturbance (n=2), multiple organ failure (n=1), and  
24 136 respiratory failure (n=1). Two hundred and forty healthy controls with no statistical  
25 137 differences in age and gender versus LC patients were selected from health  
26 138 examination population who underwent a general health checkup that included a  
27 139 physical examination and some clinical laboratory tests at the Health Care Centre of  
28 140 the First Affiliated Hospital of Medical College of Zhejiang University between  
29 141 September 2013 and October 2013. They corresponded to HBsAg negative  
30 142 individuals with normal liver function, normal renal function, and no infection. One  
31 143 hundred and thirty-four CHB patients and 240 healthy controls were used to compare  
32 144 basic characteristics with 479 LC patients.

#### 145 146 ***Ethics statement***

147 This study was approved by the Ethics Committee of the First Affiliated Hospital of  
148 the Medical College at Zhejiang University in China and was performed in  
149 accordance with the Helsinki Declaration.

#### 150 151 ***Laboratory assessment***

152 All venous blood samples were obtained in the morning following a 12 h fast, within  
153 24 h after admission. All study participants were subjected to the following  
154 determinations: serum total protein (TP), albumin (ALB), total bilirubin (TB), alanine  
155 aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), total  
156 cholesterol (Tch), creatinine (Cr), prothrombin time (PT), complete blood cell counts,  
157 LMR in peripheral blood), international normalized ratio (INR) and the model for  
158 end-stage liver disease (MELD) score based on TB, Cr, INR and PT<sup>18</sup>. Complete

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3 159 blood cell counts were determined using a Sysmex XE-2100 automated hematology  
4 160 analyzer (Sysmex Corp, Kobe, Japan) with Sysmex reagents.  
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### 161 162 **Statistical analysis**

163 Statistical analysis was performed using SPSS16.0 (SPSS Inc. IL, USA). Data were  
164 presented as mean  $\pm$  SD, median (range) or categorical data as percentages, if  
165 appropriate. The differences between two groups were assessed with an independent  
166 sample t-test, the Mann-Whitney U test or chi-square test, if appropriate. Multiple  
167 comparisons were performed by one-way analysis of variance (ANOVA) or  
168 Kruskal-Wallis H tests, if appropriate. The LC cohorts were randomly divided into  
169 estimation and validation cohorts by random number generators. Spearman  
170 correlation test was used in correlation analyses. The receiver operating characteristic  
171 (ROC) curve and cutoff values of LMR were obtained, and area under ROC curve  
172 (AUROC) was calculated to identify the best LMR and/or MELD score for predicting  
173 mortality in LC patients. For AUROC analysis of combined 1/LMR and MELD score  
174 for predicting mortality in LC patients, predictive models of 1/LMR, MELD, and  
175 1/LMR + MELD were first developed by binary logistic regression analyses,  
176 respectively. Probabilities of 1/LMR, MELD, and 1/LMR + MELD were then  
177 generated, respectively, and used as three new input variables for the ROC curve  
178 analysis (shown in Figure 2). These parameters were selected by stepwise regression,  
179 and multivariate logistic regression analyses were used to evaluate if low LMR was an  
180 independent factor for predicting mortality in LC patients by an unadjusted model and  
181 adjusting for TP, ALB, TB and MELD score. The high LMR group was used as the  
182 reference category. Statistical significance was defined at  $P < 0.05$ .

## 183 184 **Results**

### 185 186 **Patient characteristics**

187 There were 479 LC patients, 134 CHB patients, and 240 healthy controls enrolled in  
188 our study. The patient characteristics are listed in Table 1. No statistical differences  
189 were observed for gender and age between the three groups. Whereas, TP, ALB, TB,  
190 ALT, AST, TG, Tch, Cr, INR, LMR, and WBC count had statistically differences (all  
191  $P < 0.05$ ). The MELD score and mortality of the LC group were statistically higher  
192 than those of the CHB group ( $P < 0.001$ ).

### 193 194 **LMR is lower in LC, especially in the non-surviving group**

195 The LMR was significantly lower in the LC group compared to the control group  
196 (2.77 vs. 5.30, respectively) and to the CHB group (2.77 vs. 3.64;  $P < 0.01$ ). The  
197 clinical characteristics and differences in variables between non-surviving and  
198 surviving LC patients are presented in Table 2. The non-surviving patients had lower  
199 LMR (Fig. 1), TP, ALB, and Tch, and higher MELD score, TB, ALT, AST, TG, Cr,  
200 INR, WBC, monocytes, and rate of decompensated cirrhosis, compared with  
201 surviving patients. The median and range of lymphocyte count of the non-surviving  
202 group were slightly lower than those of the surviving group, but the difference did not



203 reach statistical significance. These data indicate that the lower LMR in the  
204 non-surviving group was mainly due to an increased number of monocytes and  
205 secondarily due to decreased lymphocytes. LMR resulted in no significant differences  
206 in LC patients whose primary cause of death was upper gastrointestinal bleeding,  
207 hepatic encephalopathy, or hepato-renal syndrome ((1.35[0.35-17.75]),  
208 1.42[0.27-18.20], 1.39[0.39-18.25],  $p=0.955$ ).

#### 209 ***LMR is correlated to the MELD score***

210 The LMR in the LC group negatively correlated with MELD score ( $r = -0.241$ ;  $P <$   
211  $0.05$ ), especially in non-surviving LC patients, LMR negatively correlated with  
212 MELD score with a higher correlation coefficient ( $r = -0.354$ ;  $P = 0.013$ ) compared  
213 with LMR in surviving LC patients.  
214

#### 215 ***The power for predicting 1-year mortality of LMR***

216 The enrolled 479 LC patients were randomly divided into two cohorts: the training  
217 cohort ( $n = 239$ ) and the validation cohort ( $n = 240$ ). The ROC curve analyses of the  
218 training cohort were applied to estimate LMR and MELD score to predict mortality of  
219 LC patients (Fig. 2). LMR was changed into  $1/$ LMR by inverse transformation. The  
220 AUROCs of  $1/$ LMR and MELD score were 0.817 (95% confidence interval (CI):  
221 0.746 - 0.888;  $P < 0.001$ ) and 0.868 (95% CI: 0.795 - 0.941;  $P < 0.001$ ), respectively.  
222 The cutoff values, sensitivity and specificity of MELD were 19.1, 73.9 and 96.4%.  
223 LMR values were 2.10, 82.6, and 78.8%. When  $1/$ LMR and MELD score were  
224 combined, the AUC was 0.876 (95% CI: 0.808 - 0.945;  $P < 0.001$ ), only slightly  
225 higher than AUC of MELD score, and neither the specificity (71.7%) nor the  
226 sensitivity (96.9%) was significantly improved. Applying the LMR to the validation  
227 cohort, the AUROCs of  $1/$ LMR, MELD score, and  $1/$ LMR+MELD were 0.773 (95%  
228 CI: 0.692 - 0.854;  $P < 0.001$ ), 0.887 (95% CI: 0.829 - 0.945;  $P < 0.001$ ), 0.890 (95%  
229 CI: 0.836 - 0.944;  $P < 0.001$ ), respectively. There were no significant differences in  
230 the AUCs of LMR between the estimation and validation cohorts ( $Z = 0.741$ ,  $P =$   
231  $0.053$ ). To summarize, LMR was an easy parameter to achieve and the power for  
232 predicting mortality of LMR was similar to that of MELD.  
233

#### 234 ***LMR is an independent prognostic factor of mortality in multivariate analysis***

235 MELD, low LMR (LMR  $\leq 2.10$ , with high LMR  $> 2.10$  as a reference), TP, TB and  
236 ALB were selected by stepwise regression from the above parameters ( $P = 0.025$ ,  $<$   
237  $0.001$ ,  $0.048$ ,  $0.006$ ,  $< 0.001$  and  $0.021$ , respectively) with forward selection.  
238 Subsequent multivariate logistic regression analysis showed that low LMR was an  
239 independent factor for predicting mortality in LC patients (Table 3).  
240

#### 241 **Discussion**

242 In the present retrospective study of HBV-LC a significant negative association was  
243 found between LMR in the peripheral blood and the MELD score. LMR of LC  
244 patients was statistically lower, and the MELD score and mortality of LC patients  
245 were statistically higher than those of CHB and control groups, especially in the  
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3 247 non-surviving LC subgroup. Moreover, low LMR was an independent predictive  
4 248 factor of mortality. These results provide the first evidence for an association between  
5 249 LMR and mortality in LC patients.

6  
7 250 Each year approximately 2%-5% of compensated cirrhosis patients develop  
8 251 decompensation, decompensated cirrhosis patients mainly die of cirrhosis-related  
9 252 complications, and the prognosis of decompensated cirrhosis is markedly worse, with  
10 253 a 5-year survival of 14%-35% compared to 84% in compensated cirrhosis<sup>19-20</sup>.  
11 254 Decompensated cirrhosis patients frequently present with more than one facet of liver  
12 255 decompensation, and should then receive liver intense medical care and  
13 256 transplantation evaluation<sup>19</sup>. In our non-surviving group, most patients had  
14 257 decompensated cirrhosis, and their LMR values were significantly lower than those of  
15 258 the surviving group where most patients had compensated cirrhosis. LMR was  
16 259 significant correlated to the MELD score with a low ( $r$ ) correlation coefficient.  
17 260 However the  $r$  value in non-surviving LC patients was higher than in surviving LC  
18 261 patients, indicating that the LMR changes in non-surviving LC patients were more  
19 262 pronounced, which coincided with Table 3 results.

20  
21 263 Bacterial infections are an important cause of morbidity and mortality in patients with  
22 264 LC due to an impaired immune function together with an increased passage of  
23 265 bacteria from the gut (bacterial translocation [BT])<sup>4,5,21</sup>. Once infection occurs, it may  
24 266 lead to SIRS, which can cause serious complications such as severe sepsis, renal  
25 267 dysfunction, encephalopathy, coagulopathy and multiple organ failure<sup>21</sup>. SIRS occurs  
26 268 more frequently in patients with advanced cirrhosis and portal hypertension, and is  
27 269 associated with severity of liver disease and increased risk of death in LC patients<sup>4,5</sup>.  
28 270 The mortality of LC patients with infection has been reported to be more than twice  
29 271 that of patients without infection<sup>21</sup>. Monocytes are central mediators of the immune  
30 272 response and play a crucial role in the pathogenesis of liver cirrhosis. Endotoxin leads  
31 273 to monocyte activation and promotes the release of proinflammatory cytokines such  
32 274 as interleukin-1 (IL-1), IL-6, tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), and interferon- $\gamma$  into the  
33 275 serum. This release is proportional to liver disease severity. These cytokines act in an  
34 276 autocrine and paracrine fashion and result in the recruitment of inflammatory effector  
35 277 cells, such as polymorphonuclear cells<sup>21-23</sup>. The subsequent activation of nitric oxide  
36 278 (NO) *via* the cytokine cascade leads to vasodilatation<sup>24</sup>. Endotoxin, cytokines and NO  
37 279 are key elements in the pathogenesis of circulatory abnormalities in liver cirrhosis  
38 280 with infection. Li *et al.*, found that monocytes in HBV-related LC patients positively  
39 281 correlated with the endotoxin level and cirrhosis severity based on the Child-pugh  
40 282 classification, indicating that the endotoxin-driven monocyte activation was an  
41 283 important factor of SIRS and multiple organ failure<sup>25</sup>. Lee *et al.*, found that LC  
42 284 patients with hepatocellular carcinoma had a high monocyte ratio and that a  
43 285 preoperative monocyte ratio > 7% was an independent risk factor for survival after  
44 286 hepatic resection<sup>11</sup>. Immune paralysis, defined as decreased human leukocyte  
45 287 antigen-DR (HLA-DR) expression on monocytes and indicating immune dysfunction,  
46 288 was found in LC patients. HLA-DR expression is a direct marker of monocyte  
47 289 function and a protective immune response in LC patients<sup>26</sup>. Monocyte HLA-DR  
48 290 expression is significantly reduced in those patients and falls in proportion to cirrhosis

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3 291 severity<sup>27,28</sup>. Therefore, LC patients may have high monocyte count but low  
4 292 monocyte HLA-DR expression for systemic inflammatory response and immune  
5 293 paralysis. Early diagnosis and treatment of infections can significantly reduce  
6 294 morbidity and improve survival of LC patients<sup>4,5,22,24,25,29</sup>.

7  
8 295 Inflammatory stimuli mainly affect the numbers of monocytes in the peripheral blood  
9 296 in LC patients, which contributes to LMR changes. In addition, the present study  
10 297 showed that lymphocytes in the non-survival group showed a trend towards lower  
11 298 levels as compared with the survival group, without reaching statistical significance.  
12 299 Such a decline might be attributed to lymphocytopenia<sup>30,31</sup>. This is in accordance  
13 300 with Leithead *et al.*, who found that a lower lymphocyte count was associated with  
14 301 mortality in patients with end-stage cirrhosis listed for liver transplantation<sup>28</sup>.  
15 302 Lombardo *et al.*, also found that the progressive and severity-related decrease in  
16 303 peripheral blood T-lymphocyte suggested a progressive impairment of protective  
17 304 immune function in LC<sup>31</sup>. Therefore, high monocytes together with low lymphocytes  
18 305 may reflect the severity and progression of liver injury in LC patients.

19 306 LMR has been shown to be associated with tuberculosis and influenza virus infection  
20 307<sup>16,32</sup>. Recently, LMR has also been reported to predict survival and prognosis in  
21 308 various patient populations with malignant diseases<sup>12-15</sup>, and a decreased LMR has  
22 309 been shown to be significantly associated with a high risk for critical limb ischemia in  
23 310 peripheral arterial occlusive disease patients<sup>33</sup>. Compared with another novel  
24 311 inflammation index, the ability of NLR for predicting mortality (AUROC) in LC  
25 312 patients<sup>34</sup> was similar to LMR in our study. LMR was associated, in our study, with  
26 313 MELD score, the power for predicting mortality of LMR was similar to that of MELD,  
27 314 and was an independent predictive factor of mortality. In addition, the LMR is an  
28 315 easily available and low price biomarker. However, it should be noted that this was a  
29 316 retrospective study so that prospective cohorts are warranted in order to confirm the  
30 317 present data. Another study limitation was a 1:1 ratio was not adopted for setting up  
31 318 the control groups. Moreover, these findings may only apply to HBV-related LC  
32 319 patients and, therefore, need to be validated in other etiologies of liver cirrhosis by  
33 320 future prospective clinical trials.

34 321 **Contributorship statement** L.F. designed the experiments. G. F., JW.Z. J.Z  
35 322 performed the experiments. Y.Z. and J.Y. analyzed and interpreted all the data. J.Z and  
36 323 Y.Z. wrote the main manuscript text. All authors reviewed the manuscript.

37 324 **Competing interests** None declared.

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42 329 **Ethics approval** This study was approved by the ethics committee of the First  
43 330 Affiliated Hospital of Zhejiang University School of Medicine, China. Patient consent  
44 331 Obtained.

45 332 **Data sharing statement** No additional unpublished data are available.

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335

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## Figure Legends

**Figure 1:** The boxplots of MELD score and LMR between surviving and non-surviving LC patients  
LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score.

**Figure 2:** Receiver operating characteristic (ROC) curve analysis for predicting mortality by LMR and MELD score in the training cohort.  
LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score; 1/LMR+MELD, 1/LMR combined with MELD.

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**Table 1.** Basic characteristics of enrolled participants.

Variables	Control (240)	CHB (134)	LC (479)	<i>P</i> value
Female/male	61/179	34/100	126/353	0.956
Age (year)	50.6±9.69	48.9±8.04	50.8±10.8	0.163
HBsAg positive (yes/no)	0/240	134/0	479/0	-
HBeAg positive (yes/no)	0/240	66/68	184/295	0.024 <sup>#</sup>
TP (g/L)	71.6±3.79	67.3±6.83*	62.9±8.48* <sup>#</sup>	<0.001
ALB (g/L)	46.2±3.17	37.4±5.95*	33.2±5.61* <sup>#</sup>	<0.001
TBIL (μmol/L)	12(6-49)	21.5(5-309)*	31(5-839)* <sup>#</sup>	<0.001
ALT (U/L)	17(7-48)	61(9-1838)*	29(4-1882)* <sup>#</sup>	<0.001
AST (U/L)	19(12-46)	48(16-1235)*	40(8-4094)* <sup>#</sup>	<0.001
TG (mmol/L)	1.08(0.41-1.70)	1.33(0.44-4.14)*	0.79(0.3-3.59)* <sup>#</sup>	<0.001
Tch (mmol/L)	4.66(2.40-5.86)	4.04(1.6-8.17)*	2.89(0.74-9.73)*	<0.001
Cr (μmol/L)	73(39-100)	65(29-154)*	66(30-729)*	0.002
INR	0.94±0.05	1.21±0.23*	1.55±0.78* <sup>#</sup>	<0.001
WBC (10 <sup>12</sup> /L)	5.6(4.0-9.4)	4.75(2-12)*	3.9(0.8-32.8)* <sup>#</sup>	<0.001
LMR	5.30(1.4-13.2)	3.64(0.65-9.61)*	2.77(0.27-18.25)* <sup>#</sup>	<0.001
MELD score	-	5.89(0-23.63)	9.89(0-57.17)	<0.001 <sup>#</sup>
Mortality (yes/no)	-	1/133	92/387	<0.001 <sup>#</sup>

Data were presented as mean ± SD and median (range). CHB, chronic hepatitis B; LC, liver cirrhosis; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; TP, total protein; ALB, Albumin; TB, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; Tch, total cholesterol; Cr, creatinine; INR, international normalized ratio; WBC, white blood cell; LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score. *P*-value: Comparison among these three groups. <sup>#</sup>: LC group vs. CHB group. \*: *P* < 0.05 vs. the Control group



**Table 2.** The clinical characteristics and differences in variables between non-surviving and surviving LC patients.

Variables	Non-surviving (n=92)	Surviving (n=387)	<i>P</i> value
Female/male	30/62	96/291	0.127
Age (year)	53.8±10.3	50.1±10.8	0.003
TP (g/L)	56.4±8.40	64.5±7.74	<0.001
ALB (g/L)	29.7±5.17	34.0±5.40	<0.001
TBIL (µmol/L)	292.5(9-839)	27(5-836)	<0.001
ALT (U/L)	48(4-1882)	27(5-475)	<0.001
AST (U/L)	66(10-4094)	37(8-440)	<0.001
TG (mmol/L)	0.88(0.30-2.15)	0.76(0.33-3.59)	0.022
Tch (mmol/L)	1.83(0.74-5.29)	3.02(0.94-9.73)	<0.001
Cr (µmol/L)	73.5(30-729)	65(30-326)	<0.001
INR	2.23±1.51	1.39±0.28	<0.001
WBC (10 <sup>9</sup> /L)	6.75(0.8-24.9)	3.6(0.9-32.8)	<0.001
Monocytes(10 <sup>9</sup> /L)	0.73 (0.04-3.16)	0.33 (0.05-2.0)	<0.001
Lymphocyte(10 <sup>9</sup> /L)	0.9 (0.1-4.3)	1.00 (0.10-5.40)	0.166
LMR	1.41(0.27-18.25)	3.10(0.38-14.58)	<0.001
MELD score	22.94(0.84-57.17)	8.49(0-35.33)	<0.001
Decompensated cirrhosis (yes/no)	82/10	26/361	<0.001

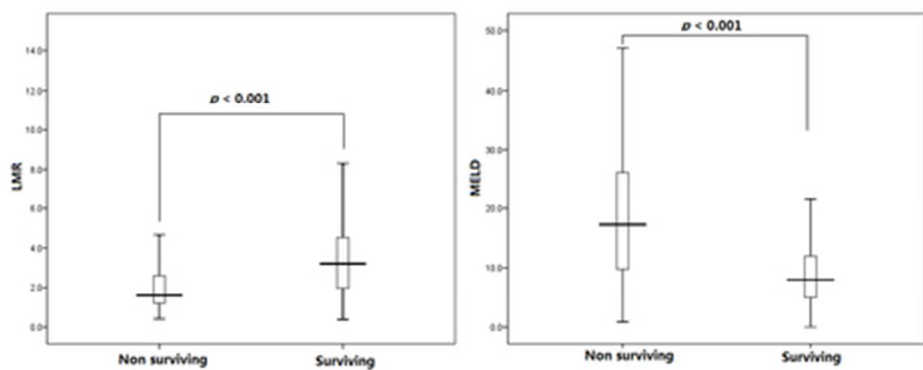
Data were presented as mean ± SD and median (range). LMR, lymphocyte to monocyte ratio; LC, liver cirrhosis; TP, total protein; ALB, Albumin; TB, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; Tch, total cholesterol; Cr, creatinine; INR, international normalized ratio; WBC, white blood cell; MELD score, model for end-stage liver disease score.

**Table 3.** Odds ratios of low LMR for predicting mortality in LC patients

Models	Odds Ratio (95% CI)	<i>P</i> value
Model 1	8.623 (5.051-14.721)	< 0.001
Model 2	3.324 (1.571-7.035)	< 0.001
Model 3	2.370 (1.070-5.249)	0.033

Odds ratios of low LMR were determined using high LMR as reference; model 1: unadjusted; model 2: adjusted for TP, ALB, and TB; model 3: adjusted for TP, ALB, TB and MELD score.

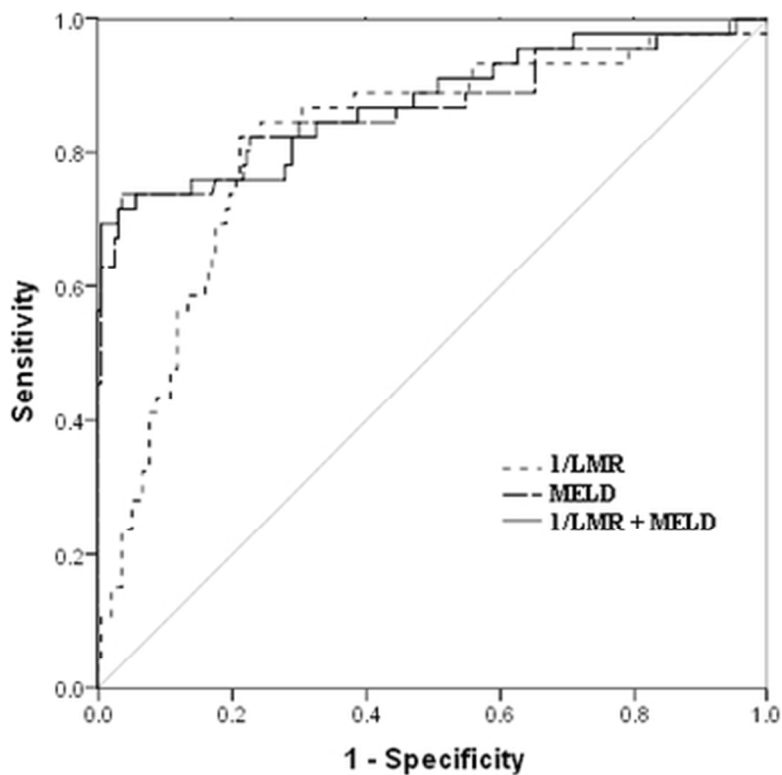
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**STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology\***  
**Checklist for cohort, case-control, and cross-sectional studies (combined)**

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	4-5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	no
Study size	10	Explain how the study size was arrived at	no
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	5-6
		(b) Describe any methods used to examine subgroups and interactions	5-6
		(c) Explain how missing data were addressed	no
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	5-6

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		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	5-6
<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5,table 1
		(b) Give reasons for non-participation at each stage	no
		(c) Consider use of a flow diagram	no
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5,table 1
		(b) Indicate number of participants with missing data for each variable of interest	no
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	no
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	5-6,table 1-2
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6,table 3
		(b) Report category boundaries when continuous variables were categorized	6
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	6-7,table 3
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8-9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7-8
Generalisability	21	Discuss the generalisability (external validity) of the study results	7-9
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).